Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 5, No. 2, p. 562-571, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

# Inhibition of persian walnut (*Juglans regia* L.) microcuttings browning by utilizing different methods

**Abdollah Ehteshamnia*, Mansour Gholami**

*Department of Horticultural Sciences, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran*

Article published on August 24, 2014

**Key words:** activated charcoal, ascorbic acid, browning, polyvinyl pyrrolidone, subculture.

**Abstract**

Microcuttings browning and eventual death of the tissue during the initial stage of walnut tissue culture is a frequent problem. In this research, three different experiments were conducted to inhibition microcuttings browning. In first experiment, the effect DKW, DKW and MS culture medium fortified with activated charcoal (AC) and ascorbic acid (AA) on the initiation medium for two Persian walnut cultivars chandler and Jamal were evaluated. Results of this experiment indicated that DKW medium fortified with AC were optimum medium than MS and DKW Supplemented with AC and AA. In second experiment, the subculturing thrice of two walnut cultivars microcuttings at an interval of 48 hrs in DKW basal medium and DKW medium containing AC and AA were studied. The microcuttings subculturing in DKW medium containing AC was better than subculturing in DKW medium containing AA but there was no significant difference between them. In third experiment, the microcuttings were soaked for 2 h before culture by following solutions: soaked in distilled water, polyvinyl pyrrolidone (PVP) and AA for two walnut cultivars. Microcuttings soaked in PVP had suitable establishment than AA and distilled water. Finally, this research indicated in most cases successful control of Persian walnut microcuttings browning could be achieved by different combinations of these methods.

***Corresponding Author:** Abdollah Ehteshamnia ✉ ab.ehteshamnia@gmail.com

## Introduction

Walnut tree (*Juglans regia* L.), belongs to family Juglandaceae, is an important temperate nut crop. Walnuts rank third in nut production after cashews and almonds (FAOSTAT, 2011). During the last decade, the worldwide walnut production was doubled, probably reflecting on the increase in consumers demand for this nut (Christopoulos and Tsantili, 2011). The first attempts at walnut micropropagation utilized existing medium formulations which were suitable for other woody plants. Because woody plants are still often very difficult to culture; many different types of medium have been employed. In previous researches done at walnut micropropagation, different culture medium have been used, such as Driver and Kuniyuki (DKW) (Driver and Kuniyuki, 1984), Murashige and Skoog (MS) (Murashige and Skoog, 1962), B5 (Gamborg *et al*., 1968), Cheng (C) (Cheng, 1978), NGE (Sanchez-Zamora, 2006) and woody plant medium (WPM) (Lloyd and McCown, 1981) with varying results. Micropropagation has the immense advantage of rapidly generating a large number of genetically identical plants in a much shorter time than could be achieved by conventional propagation methods. But these techniques receive a set back by certain physiological processes which hinders the success of new technique, particularly in perennial fruit crops.

Explants browning and eventual death of the tissue during the initial stage of walnut culture is a frequent problem. One of the major obstacles associated with *in vitro* multiplication of walnut mature material is the phenolic compound exudation from the cut surface of the explants. Oxidation of these compounds caused lethal browning of explants and culture medium. Oxidation of phenolic compounds released from the cut ends of explants by polyphenoloxidases, peroxidases cause lethal browning of explants and culture medium (Bhat and Chandel, 1991). In some species the establishment of explants frequently requires special procedures to escape or avoid problems associated with oxidation of polyphenols (Ashutosh *et al.,* 2003). Difficulties reported on tissue culture of *Juglans* species were mainly in the initiation phase due to the detrimental effect of phenolic browning beside the low multiplication rate and cultures decline in the proliferation phase. Supplementing the initial medium with different additives that can prevent the production of phenolics or can remove inhibitory phenolic substances from the medium, such as antioxidants, chelate-forming materials or adsorbents (Block and Lankes, 1996; Pan and van Staden, 1998; Dobránszki *et al*., 2000 a,b,c; Sharma *et al*., 2000; Thomas, 2008). In this study, efficient shoot multiplication method from nodal segments of walnut (*Juglans regia* L.) using different nutrient medium, antioxidants, absorbent, different pretreatments and subculturing of microcuttings were evaluated. This study aimed to counteract and declining browning to enhance growth and multiplication of cultures. This research was made to control browning of cultures to get successful establishment of two Persian walnut microcuttings.

## Materials and methods

*Plant Materials*

Newly grown shoots of two Persian walnut cultivars (*Juglans regia* L.), `Chandler' and `Jamal' were collected in early May 2013 from selected mature trees growing in Walnut Research Station in Toyserkan, Iran. Trees were managed in terms of nutrition, pruning, irrigation, pests and diseases, similarly.

*Sterilization of microcuttings*

Shoots were cut into nodal segments (uninodal microcuttings with a length between 3 and 5 cm). Then were washed with tap water for 20±05 min and surface-disinfected by immersion in 70% (v/v) ethanol/water solution for 30 s followed by 1.5% (w/v) sodium hypochlorite fresh solution with two drops of Tween 20 per 100 ml for 20 min, followed by three rinses in sterile deionized water.

In this research, three different experiments have been conducted: